# September

## *Week 1 (05/09/16 - 09/09/16)*

- MODULE 1: Characterization essay of Lambda Lysozyme cassette

**Table 3.** OD results for the characterization essay.

| | Original |
|---|---|
| | Single Blank |
| Sample | OD |
| 1 | 2.61 |
| 2 | 2.309 |
| 3 | 2.075 |
| 4 | 3.077 |
| 5 | 1.767 |

For the characterization essay the concentration of the dilutions were changed in order to get better results. The change in the concentrations worked as it can be observed in the OD analysis results, we can see a greater reduction in the OD as the proteic extract increases in the dilutions. In the 4th sample with an OD of 3.077 this variation is probably caused by a mistake in the preparation of the proteic extracts, since the pellet found in the first centrifugation was not correctly resuspended.

For alternative 2, antibiograms were made in LB + CAM dishes using J04450 in DH5alpha. It can observed inhibition halos, however they are not seen in every concentration, for this reason the assay will be repeated at different concentrations starting from the ones that showed promising results. One of the most promising concentrations was 500 + 500 (Figure 5, top-right) and 200 + 600 (Figure 5, middle-left)

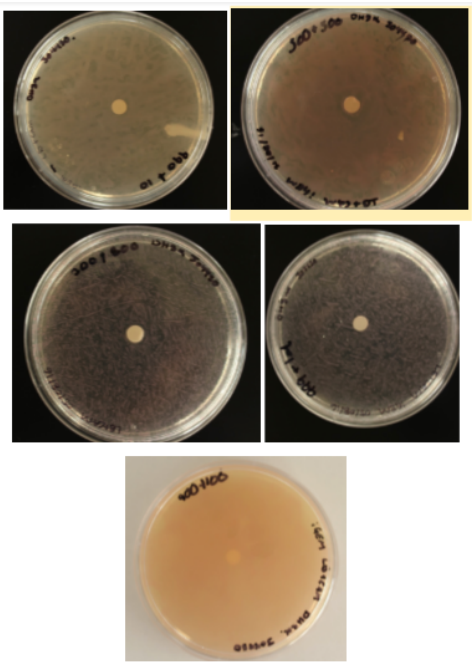

**Fig. 5** Petri dishes of R0-lambda characterization essay, alternative 2. Antibiograms with different dilutions plated in Petri Dishes with J04450 in DH5aplha in LB+CAM medium.

- MODULE 2 :Transformation into competent cells of Fused protein Endolysin - holin.

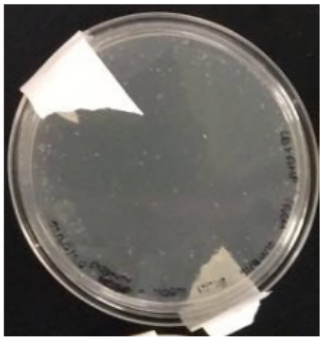

**Fig. 6** Transformation of R0011 + NNQW in a LB + AMP medium.

Three ligations were performed and transformed into competent cells but unfortunately the only one that showed proper growth was R0011 + NNQW (Fused protein Endolysin - holin) in BL21, however, since none of the DH5alpha transformations grew, the process will be repeated with comercially available competent cells.

Summary:
The ligation and transformation of NNQW (Fused protein Endolysin - holin) with the promoter R0011 was obtained in protein expression strain (BL21). Further stria culture and documentation (either extraction and restriction, or PCR / colony PCR) are needed to guarantee the presence of the part and start with the production and purification of the protein. Other parts are still to be ligated to both pSB1C3 and R0011 to start the production process.

The characterization showed promising results regarding OD 600 measurements that follow the expected trend of decreasing OD with increasing protein extract. Dilutions are to be made more concentrated to guarantee better growth inhibition and quantifiable inhibition when performing the alternative 2 of the characterization process.

# *Week 2 (12/09/16 - 16/09/16)*

Characterization of Lambda Lysozyme cassette, NNQW (Fused protein Endolysin - holin) colony PCR

- MODULE 1: Colony PCR of NNQW (Fused protein Endolysin - holin)

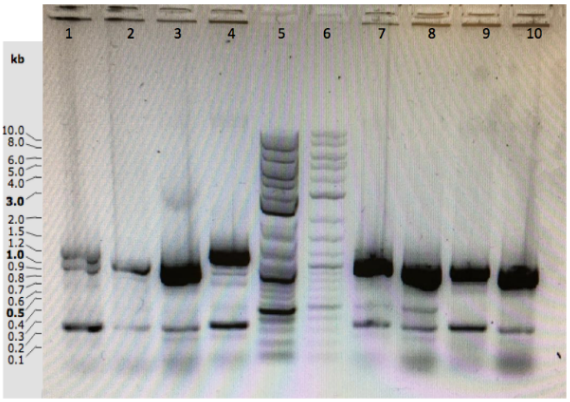


**Figure 4.** Colony PCR´s. Gel at 8% run at 100V for 50 min. 1)R0011-NNQW colony 1.1 5µL + 4µL LB. 2) R0011-NNQW colony 1.2 5µL + 4µL LB. 3)R0011-NNQW colony 2.1 5µL + 4µL LB. 4) R0011-NNQW colony 2.2 5µL + 4µL LB. 5)2 log DNA ladder 2µL. 6) 2 log DNA ladder 2µL + 4µL LB. 7) R0011-NNQW colony 2.3 5µL + 4µL LB. 8) R0011-NNQW colony 2.4 5µL + 4µL LB. 9) R0011-NNQW colony 3.1 5µL + 4µL LB. 10) R0011-NNQW colony 3.2 5µL + 4µL LB.

Regarding the Colony PCR´s performed (Figure 4) the expected migration size of the amplicons was 1693 bp, but the migration size of the amplification was around 1200 bp a possible explanation of this phenomena could be the quantity of sample, when the quantity of sample is massive it tends to be observed as if it migrated more (a clear example can be observed in the Bp markers in the fifth and sixth lanes), to confirm or discard the presence of the desired plasmid is necessary to perform a restriction analysis or a plasmid extraction.

- MODULE 2: Characterization of Lambda Lysozyme cassette

The characterization process obtained mixed results. The antibiogram assay generated inhibition but even the LB control showed inhibition, which makes the whole assay doubtful. Still the more the protein extract was added, the bigger the inhibition in some of the plates. Not all papers showed the same inhibition with the same concentration of protein extract. In general the trend was present but not consistent. The results are contrary to those registered on the absorbance lectures, were only one did not follow the trend. This problem can be solved with a duplicate of the assay for each protein extract. Still the control medium showed mixed results since it was expected to have a higher optical density than any of the ones with protein extract. It is possible to have used the LB control with different conditions, leading to the decrease of the absorbance lecture. It is also probable that the 250µL protein extract is not enough to cause a visible change in OD, due to the exponential growth of the bacteria. This would lead to very similar OD readings between the first assay and the control.

# *Week 3 (19/09/16 - 23/09/16)*

- MODULE 1: Colony PCR method of NNQW (Fused protein Endolysin - holin)

Both PCRs performed with the Q5 High Fidelity Master 2X Master Mix showed mixed results. Controls were set on both occasions to see the functionality of the master mix. For the second PCR program a negative control containing no plasmid (no colony in this case) was used along with a positive control consisting of RFP J04450, taken from a colony that visually was pink in the transformation plate, meaning that the protein was already produced and the plasmid and part were present. On both PCRs there was plasmid in the negative control, which should not appear because no plasmid was added. The same bands appeared on the following PCRs without the expected 1639bp band related to R0011-NNQW. PCR from colony 6 shows no contamination but also seems like if the well had not been loaded. All loading conditions were reviewed and the well information is confirmed for each gel (all wells were correctly loaded). The positive control showed the same band as the negative, stria 4 and stria 5 PCRs. This means that one common reagent was contaminated. On the first PCR with the Q5 Master Mix the gel showed the same 1200bp band as the one seen in the second methodology.

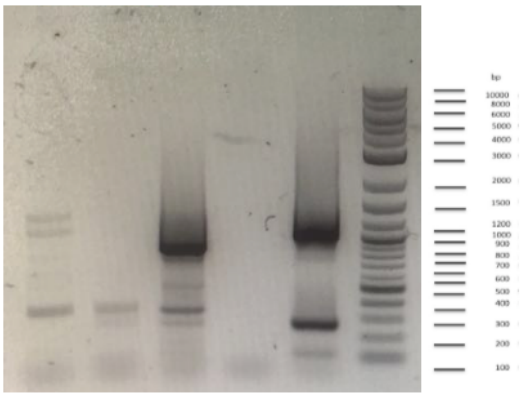

**Figure 1.** Colony PCR. Gel 0.8% agarose run at 100V for 50min. R0011-NNQW PCR product has an expected molecular weight of 1639bp. 1)Negative control PCR (no colony) 5µL + 4µL, 2) Colony PCR from stria 4 - 5µL + 4µL, 3) Colony PCR from stria 5 - 5µL + 4µL, 4) Colony PCR from stria 6 - 5µL + 4µL, 5) Positive control (Colony PCR from J04450 - RFP - 5µL + 4µL, 6) 2-log DNA Ladder - 2µL + 4µL.

Summary:
Due to the inconclusive results and the high probability of contamination in the reagents, it can be concluded that it is necessary to repeat the PCRs with new reagents in order to obtain significant and trustworthy results.